A New Intestinal Model for Analysis of Drug Absorption and Interactions Considering Physiological Translocation of Contents

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ABSTRACT

Precise prediction of drug absorption is key to the success of new drug development and efficacious pharmacotherapy. In this study, we developed a new absorption model, the advanced translocation model (ATOM), by extending our previous model, the translocation model. ATOM reproduces the translocation of a substance in the intestinal lumen using a partial differential equation with variable dispersion and convection terms to describe natural flow and micromixing within the intestine under not only fasted but also fed conditions. In comparison with ATOM, it was suggested that a conventional absorption model, advanced compartmental absorption and transit model, tends to underestimate micromixing in the upper intestine, and it is difficult to adequately describe movements under the fasted and fed conditions. ATOM explains the observed nonlinear absorption of midazolam successfully, with a minimal number of parameters. Furthermore, ATOM considers the apical and basolateral membrane permeabilities of enterocytes separately and assumes compartmentation of the lamina propria, including blood vessels, to consider intestinal blood flow appropriately. ATOM estimates changes in the intestinal availability caused by drug interaction associated with inhibition of CYP3A and P-glycoprotein in the intestine. Additionally, ATOM can estimate the drug absorption in the fed state considering delayed intestinal drug flow. Therefore, ATOM is a useful tool for the analysis of local pharmacokinetics in the gastrointestinal tract, especially for the estimation of nonlinear drug absorption, which may involve various interactions with intestinal contents or other drugs.

SIGNIFICANCE STATEMENT

The newly developed advanced translocation model precisely explains various movements of intestinal contents under fasted and fed conditions, which cannot be adequately described by the current physiological pharmacokinetic models.

Introduction

Oral formulations are commonly used in pharmacotherapy because they can deliver medicinal ingredients safely in the body and can be prescribed to outpatients (Homayun et al., 2019). However, drug absorption is seriously affected by many factors, such as disintegration of the formulation, the solubility and stability of the drug, interactions with intestinal contents, active efflux by transporters such as P-glycoprotein (P-gp), and metabolism by CYP3A (Mayer et al., 1996; Lu et al., 2017). For analysis of intestinal drug absorption, various pharmacokinetic models such as the compartmental absorption and transit (CAT) model (Yu and Amidon, 1999); advanced compartmental absorption and transit (ACAT) model (Agoram et al., 2001; Huang et al., 2009); advanced dissolution, absorption, and metabolism (ADAM) model (Jamei et al., 2009); segregated-flow model (Cong et al., 2000; Pang and Chow, 2012); QGut model (Gertz et al., 2010); gastrointestinal transit absorption (GITA) model (Haruta et al., 2002; Kimura and Higaki, 2002); and translocation model (TLM) (Ando et al., 2015) have been reported. Of these, the QGut model is a simple model using the parameter QGut and has provided adequate predictions of observed intestinal availability (FIA) values (Gertz et al., 2010); however, its application to nonlinear absorption has yet to be reported. Conversely, more advanced models, such as CAT, ACAT, or ADAM, explain the heterogeneity of the gastrointestinal tracts using multiple compartments. Each compartment possesses metabolism and transport clearances, enabling reasonable simulation of time- and location-dependent drug absorption. These models have succeeded in predicting gastrointestinal drug...
absorption, including nonlinear pharmacokinetics (Bolger et al., 2009; Takano et al., 2016).

It is noteworthy that, for these sophisticated models, multiple scaling factors are required to fill the gaps between in vitro and in vivo data. For example, scaling factors such as for the absorption surface area in each intestinal site (Hendriksen et al., 2003), $V_{max}$, and $K_m$ of metabolic enzymes or transporters are applied to predictions (Takano et al., 2016). However, the function of the scaling factors would deviate from the concept of a physiologically based pharmacokinetic (PBPK) model when the movement of the drug to each section of the small intestine is not consistent with assumptions of the model.

This problem may lie with the structures of these models that explain the translocation of a drug in the lumen from upstream to downstream. Since translocation of a drug is explained via successive first-order kinetics in CAT, ACAT, and ADAM, the degree of mixing is always increasing; thus, mixing tends to be underestimated upstream and overestimated downstream, leading to overestimation of drug concentrations upstream. For substrate drugs of metabolic enzymes or transporters, drug concentrations need to be estimated accurately at each intestinal site to consider potential nonlinear pharmacokinetics. In addition, it is necessary to include the appropriate description of blood flow in the capillary in these models. To overcome this issue, the segregated-flow model has been proposed to consider divisions of blood flow into the mucosa and submucosa (Cong et al., 2000; Pang and Chow, 2012).

Previously, we developed the TLM to solve these problems (Ando et al., 2015). To minimize the calculation load, the TLM has only one compartment for absorption, but its properties of movement are time-dependent and arbitrary. However, it is theoretically difficult to accommodate for interactions with other drugs or various contents in the gastrointestinal tract. Therefore, in this study, we constructed an advanced translocation model (ATOM) that describes drug movements in the lumen by dispersion and convection terms while maintaining the features of TLM. Dispersion models based on partial differential equations have been used in the field of local pharmacokinetics to explain drug clearances (Roberts and Rowland, 1986) and extended to nonlinear pharmacokinetics (Hisaka and Sugiyama, 1998). Here, we analyzed drug movements in the gastrointestinal tract using a dispersion model in ATOM and compared the results with those of the CAT model.

Materials and Methods

Construction of ATOM. The structure of ATOM and descriptions of the parameters are shown in Fig. 1 and Table 1, respectively. The source codes of ATOM used for the analysis were attached in the Supplemental Material. The esophagus, stomach, cecum/colon, and portal vein were expressed as separate compartments. The intestinal lumen was expressed as a one-dimensional dispersion model with a location-dependent dispersion number and time-dependent clearance. The movements of drugs, water, and intestinal contents were assumed to be the same in the lumen and thus substance-independent since micromixing and convection occur as a result of intestinal motility. In addition to these tissues responsible for drug absorption, compartments for the portal vein and liver as well as central and peripheral blood pools for the whole body were assumed to simulate drug plasma concentration. Detailed physiologic parameters, such as pH, $P_{g,p}$, and CYP3A expressions along intestine, and differential equations for tissues other than the small intestine are shown in the Supplemental Material. Overall, the definition of ATOM is quite similar to TLM (Ando et al., 2015), other than the movements of intestinal contents, to achieve equivalent predictions of oral availability in the absence of interaction. Partial differential equations related to luminal drug movements are shown in eqs. 1 and 2.

$$\frac{\partial C_{lum, z}}{\partial t} = D_z \frac{\partial^2 C_{lum, z}}{\partial z^2} - \frac{M_t}{C_{lum, z}} - \frac{f_{lum} C_{lum, z}}{X_{water, z} + V_{lum, z}}$$

$$+ \frac{PS_{a, out, z} f_{ent} C_{ent, z}}{V_{lum, z}} (z = 0, 1).$$

(1)

**Boundary condition:**

$$C_{lum, z} = \frac{D_z}{M_t} \frac{\partial C_{lum, z}}{\partial z} = \frac{k_{in} V_{in}}{V_{lum, z}} (z = 0)$$

$$\frac{\partial C_{lum, z}}{\partial z} = 0 (z = 1).$$

(2)

In these equations, length is expressed as a ratio of volume until the location to the full volume of the intestinal lumen ($V_{lum}$). $f_{ent}$ and $f_{ent, z}$ are drug concentrations in the lumen and enterocytes at location $z$ (i.e. within a small interval around $z$, the same will be applied hereafter), respectively. $D_z$ and $M_t$ represent the dispersion constant at location $z$ and flow rate in the lumen at time $t$, respectively. The units of $D_z$ and $M_t$ are $T^{-1}$ and $L^{1}$. As the length is normalized in eq. 1. The metrics $V_{lum, z}$ and $X_{water, z}$ represent the volume of the physical lumen and amount of inflating water (that may be drunk, secreted, and absorbed) at location $z$, respectively. The effective volume in the lumen used for calculation of the drug concentration to know absorption is $V_{lum, z} + X_{water, z}$, but it was assumed that $X_{water, z}$ does not affect the micromixing and flow rate. The amount of inflating water in the gastrointestinal tract was simulated with a distinct partial differential equation by considering the water secretion and absorption rate constants calculated using intestinal water content after drinking 240 ml of water (Mudie et al., 2014). The simulation results of the water profile in the stomach and small intestine and the optimized parameter values are shown in Supplemental Fig. 1 and Supplemental Table 1. $PS_{a, in, z}$ and $PS_{a, out, z}$ are the permeability clearance of the uptake from the lumen to the enterocytes and of the efflux from enterocytes to the lumen, respectively, via the apical membrane at location $z$. In this study, $PS_{a, out}$ is the sum of permeability clearance ($PS_{a, out, l}$) and transport clearance by $P_{g,p}$ ($PS_{a, Pgp}$) shown in the Supplemental Material. $f_{lum}$ and $f_{ent}$ represent drug unbound fractions in the lumen and enterocytes, respectively. In this study, $f_{lum}$ and $f_{ent}$ were assumed to be 1 and the same as the unbound fraction in the blood ($f_{b}$) as described in Supplemental Material, respectively. $V_{ent}$ is the volume of the enterocytes at location $z$.

Dispersion number and flow rate have been constant in general dispersion models for the analysis of local pharmacokinetics; however, drug distribution in the small intestine is quite complicated because of its structural or regional differences in motility (Sokolis, 2017). Therefore, the location-dependent dispersion number ($D_z$) and time-dependent flow rate ($M_t$) were examined in this study. They were independently optimized using the observed intestinal distribution of a nonabsorptive drug, $^{99m}$Tc-diethylenetriaminepentaacetic acid ($^{99m}$Tc-DTPA) (Haruta et al., 2002) by nonlinear least-squares method. The eqs. 3 and 4 were used for the calculation of $D_z$ and $M_t$ in both fasted and fed states.

$$D_z = A e^{x p(-B z^2)} + 0.005$$

$$M_t = C \left(1 - D \exp \left[ \frac{t - t_{lag}}{2 \sigma^2} \right] \right).$$

Where $A$, $B$, $C$, $D$, $E$, and $F$ are the adjusting constants, $t$ is the time after administration, and $t_{lag}$ is the time at the minimum flow rate. Parameters $A$–$E$ and $t_{lag}$ were optimized simultaneously using the observed $^{99m}$Tc-DTPA distribution in the lumen reported by Haruta et al. (2002). The parameter $F$ was fixed to 4 in this study. Among models that fixed for both $D_z$ and $M_t$, variable $D_z$ but fixed $M_t$ and variable $D_z$ and $M_t$, the best model was selected based on the Akaike’s information criterion value.

Regarding perpetrators of DI, drug concentrations of the perpetrator in tissues were simulated with partial differential equations in the intestine (eqs. 1–4) and differential equations in other tissues (shown in Supplemental Material) as well as substrates. Intestinal clearance changes of $P_{g,p}$ transport and CYP3A metabolism upon administration of a perpetrator were calculated using eqs. 5 and 6 with unbound drug concentration of a perpetrator and inhibition constant for P-
gp ($K_{i,Pgp}$) and CYP3A ($K_{i,CYP3A}$).

$$PS_{a,Pgp,z} = \frac{PS_{a,Pgp,z}}{1 + \frac{I_{out}}{K_{i,Pgp}}}$$  \hspace{1cm} (5)

$$CL_{ent,z}^* = \frac{CL_{ent,z}}{1 + \frac{I_{con}}{K_{CYP3A}}}$$ \hspace{1cm} (6)

where $PS_{a,Pgp,z}$ and $CL_{ent,z}^*$ represent active transport clearance of a substrate by P-gp and intestinal intrinsic clearance of a substrate by CYP3A, respectively, in the absence of a perpetrator (shown in Supplemental Material). $PS_{a,Pgp,z}^*$ and $CL_{ent,z}^*$ represent $PS_{a,Pgp,z}$ and $CL_{ent,z}$ values in the presence of a perpetrator, respectively. $I_{con}$ and $I_{out}$ are the unbound fraction and concentration in the enterocytes of a perpetrator, respectively.

**Analysis with CAT Model.** For comparison, CAT model with six intestinal compartments was constructed in this study (Fig. 1). The initial three compartments were for the upper intestine, and the latter were for the lower intestine. Transit times of the CAT model were optimized to fit the reported movements of $^{99m}$Tc-DTPA in the lumen (Haruta et al., 2002); original and optimized transit times are shown in Supplemental Table 3. The length of the small intestine, radius of the inlet and outlet of the intestine, CYP3A and P-gp expression profile, and pH gradient were the same as those adopted in ATOM.

**Simulation of Drug Concentrations in Enterocytes and Absorption to Portal Vein.** Drug concentration in enterocytes and accumulation of the compound in the portal vein were simulated using ATOM and CAT after oral administration ($t = 0$ hours). To predict drug concentrations in enterocytes, three time points ($t = 0.5, 2,$ and 6 hours) were selected to evaluate the drug concentrations and compare the two models. In this analysis, a compound with low permeability (non-CYP3A and non-P-gp substrate) was selected to clearly understand the difference in absorption sites
### TABLE I

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>(C_{L_{int}})</td>
<td>Intestinal metabolic clearance by CYP3A</td>
</tr>
<tr>
<td>(C_{L_{hep}})</td>
<td>Hepatic intrinsic clearance</td>
</tr>
<tr>
<td>(C_L)</td>
<td>Renal clearance</td>
</tr>
<tr>
<td>(D_0)</td>
<td>Location-dependent dispersion number</td>
</tr>
<tr>
<td>(f_b)</td>
<td>Hepatic unbound fraction</td>
</tr>
<tr>
<td>(k_{col})</td>
<td>Transit rate from the ileum to the cecum/colon</td>
</tr>
<tr>
<td>(k_{es})</td>
<td>Transit rate of drug from the esophagus to the stomach</td>
</tr>
<tr>
<td>(k_{fec})</td>
<td>Transit rate from the cecum/colon to the feces</td>
</tr>
<tr>
<td>(k_{ent})</td>
<td>Transit rate from the (n)th peripheral compartment to the central compartment</td>
</tr>
<tr>
<td>(k_{m})</td>
<td>Transit rate of drug from the stomach to the jejunum</td>
</tr>
<tr>
<td>(k_{in})</td>
<td>Transit rate from the (n)th compartment in the small intestine in CAT</td>
</tr>
<tr>
<td>(k_{trans})</td>
<td>Transit rate from the central compartment to the (n)th peripheral compartment</td>
</tr>
<tr>
<td>(M_T)</td>
<td>Time-dependent intestinal flow rate</td>
</tr>
<tr>
<td>(PS_{in})</td>
<td>Permeability clearance from the lumen to the enterocytes in the apical membrane</td>
</tr>
<tr>
<td>(PS_{ap, out})</td>
<td>Permeability clearance from the enterocytes to the enterocytes in the apical membrane</td>
</tr>
<tr>
<td>(PS_{in, lamina})</td>
<td>Permeability clearance from the enterocytes to the lamina propria in the basolateral membrane</td>
</tr>
<tr>
<td>(PS_{b, out})</td>
<td>Permeability clearance from the lamina propria to the enterocytes in the basolateral membrane</td>
</tr>
<tr>
<td>(PS_{bl})</td>
<td>Overall clearance in the basolateral membrane</td>
</tr>
<tr>
<td>(Q_b)</td>
<td>Blood flow in the lumen</td>
</tr>
<tr>
<td>(Q_m)</td>
<td>Arterial blood flow in the lumen</td>
</tr>
<tr>
<td>(Q_{pro})</td>
<td>Blood flow rate in the lamina propria</td>
</tr>
<tr>
<td>(Q_{pvl})</td>
<td>Blood flow rate in the portal vein</td>
</tr>
</tbody>
</table>

between the two models. The model compound has the same physiologic and pharmacokinetic parameters as midazolam (molecular weight, \(k_{hep}\) values, \(P_{in,CYP3A}\), and unbound fraction), except for the apparent permeability in Caco-2 cells and \(V_{max,CYP3A}\), which were set to 0.002 cm/h (approximately 1/50 compared with midazolam) and 0 \(\mu\)g/h pmol CYP3A, respectively. Regarding dispersion number and flow rate, optimized values using \(^{99m}\)Tc-DTPA distribution of one subject (subject A) in the fasted state (shown in Fig. 2A and Supplemental Table 2) were used. Dose was set to 1 ng.

#### Prediction of Nonlinear Absorption of Midazolam and Unbound Concentration in Enterocytes in Both Fasted and Fed States

Dose-dependent absorption ratio \((F_AF_G)\) and concentrations in enterocytes with regard to typical CYP3A substrate (midazolam) were simulated for 4 hours after oral administration using ATOM and CAT. In simulations by ATOM, \(F_AF_G\) values of midazolam were predicted in the fasted and fed states, with the optimized dispersion constant and flow rate obtained using \(^{99m}\)Tc-DTPA distribution of one subject A (Haruta et al., 2002), as shown in Fig. 2. It was confirmed that intestinal absorption of midazolam was completed at 4 hours after oral administration. \(F_AF_G\) values were calculated using eq. 7:

\[
F_AF_G = \frac{X_{cumulative}}{Dose},
\]

where \(X_{cumulative}\) represents accumulated drug amount in the portal vein.

Reported \(F_AF_G\) values were obtained from previous reports (Ando et al., 2015; Bornemann et al., 1986). Pharmacokinetic parameters of midazolam and physiologic parameters used in these models are shown in Supplemental Tables 4 and 5. To predict unbound drug concentration in enterocytes \((C_{u, in})\), simulated results at 0.16 hours after administration, at which maximum concentration was observed, were used.

#### Comparison of \(F_G\) Predicted Values between ATOM and TLM

\(F_G\) prediction by ATOM was performed using the same data set used in the previous report of TLM (Ando et al., 2015), and \(F_G\) values between ATOM and TLM were compared. In the simulation by ATOM, simulation results at 4 hours after oral administration are shown because intestinal absorption was completed. \(F_G\) was calculated from absorption ratio \((F_A)\) and \(F_AF_G\) using the following equations (eqs. 8 and 9):

\[
F_A = \frac{X_{cumulative}}{Dose}
\]
\[
F_G = \frac{F_AF_G}{1 + \frac{k_{col}}{k_{ent}}}
\]

where \(X_{cumulative}\) represent accumulated drug amount in the portal vein. In the prediction of \(F_G\) values, optimized values using \(^{99m}\)Tc-DTPA distribution of subject A in the fasted state (shown in Fig. 2A and Supplemental Table 2) were used.

#### Simulation of DI Mediated by CYP3A and P-gp Using ATOM

Regarding CYP3A-mediated DI simulation, reported plasma concentrations of midazolam with itraconazole (a typical CYP3A inhibitor) were used (Templeton et al., 2010). Briefly, 0, 50, 200, and 400 mg itraconazole were administered \((t = 0\) hours) 4 hours before midazolam administration, and then 2 mg midazolam was administered \((t = 4\) hours). Regarding P-gp-mediated DI simulation, reported plasma concentrations of digoxin with clarithromycin (a typical P-gp inhibitor) were used (Rengelshausen et al., 2003). Briefly, 250 mg clarithromycin was administered \((t = 0\) hours) twice a day. At 24 hours after the first administration of clarithromycin, 0.75 mg digoxin was administered \((t = 24\) hours). The plasma concentrations of midazolam and digoxin were simulated for 24 hours after oral administration of the substrate in the presence or absence of a perpetrator according to the reports by Templeton et al. (2010) or Rengelshausen et al. (2003). DI simulation was performed using optimized values using \(^{99m}\)Tc-DTPA distribution of subject A in the fasted state (shown in Fig. 2A and Supplemental Table 2). In this simulation, only the intestinal contribution to the DI was considered; thus, hepatic and renal inhibitions of the metabolizing enzyme or transporter were not included. The pharmacokinetic parameters of these drugs and the physiologic values used in these analyses were obtained from previous reports or results using ADMET Predictor and GastroPlus (Simulations Plus, Inc.), shown in Supplemental Tables 4 and 5. Two pharmacokinetic parameters \((f_p\) for digoxin and \(K_{Pgp}\) for clarithromycin) were obtained from the printed labeling of Digoxin Elixir (Roxane Laboratories, Inc.; available online: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/021648s000_PRNTLBL.pdf) and the pharmacology reviews of PRADAXA (dabigatran etexilate mesylate) Capsules (Boehringer Ingelheim Pharmaceuticals, Inc.; available online: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2010/022512Orig1s0000pharmR_Corrected@203.11.2011.pdf), respectively. Body weight was assumed to be 70 kg for calculating pharmacokinetics parameters.

#### Simulation of Plasma Concentration after Oral Administration of Midazolam and Digoxin in the Fasted and Fed States

Plasma concentration of midazolam and digoxin in the fasted and fed states were simulated for 24 hours after oral administration. Dose was set to 2 mg (midazolam) and 0.75 mg (digoxin), respectively. In these simulations, the dispersion constant and flow rate in the intestine obtained using \(^{99m}\)Tc-DTPA distribution of subject A in the fasted state (shown in Fig. 2A and Supplemental Table 2) were used. Body weight was assumed to be 70 kg for calculating pharmacokinetics parameters.

#### Calculation of Dispersion Model

The model with three nonlinear partial differential schemes (substrate, perpetrator, and inflating water) was solved by finite difference method (Hisaka and Sugiyama, 1998), with differential schemes for the associated organs (esophagus, stomach, colon, portal vein, liver, central and peripheral compartments of the body). The number of segments was determined to be 40 considering the precision of the calculation. The Danckwerts’ (closed) boundary conditions were implemented. Schemes for all the segments and compartments were calculated simultaneously with the Runge-Kutta-Fehlberg method using Numeric Analysis Program for Pharmacokinetics (version 2.31) (Hisaka and Sugiyama, 1998). The parameter fitting was performed mainly by the quasi-Newton method with the Broyden-Fletcher-Goldfarb-Shanno scheme implemented in Numeric Analysis Program for Pharmacokinetics.

#### Results

Determination of Dispersion Number in ATOM and Application to the Simulation of \(^{99m}\)Tc-DTPA Distribution in the Lumen Considering Effect of Food

ATOM explains movements of intestinal contents, including the target drug, by the dispersion model with potentially variable dispersion number and flow rate. Thus, these terms need
Fig. 2. Simulated movements of $^{99m}$Tc-DTPA in the gastrointestinal tract by ATOM (A–D) and CAT (E–H) in fasted and fed states. The observed $^{99m}$Tc-DTPA distribution in the lumen was taken from a previous study (Haruta et al., 2002), which reported its distribution in two subjects. Simulated results of $^{99m}$Tc-DTPA distribution of subject A in the fasted condition (A and E), subject A in the fed condition (B and F), subject B in the fasted condition (C and G), and subject B in the fed condition (D and H). The left (A–D) and right (E–H) panels show the simulated results by ATOM and CAT, respectively. Open circles, closed circles, open squares, and open triangles represent the observed distributions in the stomach, upper intestine, lower intestine, and cecum/colon, respectively. The lines represent the simulated $^{99m}$Tc-DTPA distributions in each organ. In ATOM, distributions at 40 locations were simulated: the upper 24 segments were assigned to the upper intestine, and the other 16 segments were assigned to the lower intestine, corresponding to the CAT model using the index of the length from the inlet in the intestine. The CAT model assumes that the small intestine is divided into six portions, as shown in Fig. 1.
to be determined to reproduce the observed movements of intestinal contents. In the fasted state, nonabsorbable $^{99m}$Tc-DTPA filled the upper jejunum within 2 hours after dosing and, thereafter, rapidly moved to the lower jejunum, where it was retained for the next 2 hours, according to the report by Haruta et al. (2002) (Fig. 2, A, C, E, and G). In the fed state, in addition to delayed gastric emptying, movement of the contents slowed, and a part of it was retained in the upper jejunum even 3 hours after dosing (Fig. 2, B, D, F, and H). The model with a location-dependent variable dispersion number successfully reproduced the movements of the rapid passage through the upper jejunum and the subsequent retention in the lower jejunum, but only for one of the two fasted subjects (Figs. 2A and 3A). The model with a fixed dispersion term failed to reproduce the movement even for this subject (Supplemental Fig. 2). For the remaining observations, including in the fed state, a model with time-dependent flow rate, in addition to the location-dependent dispersion number, was necessary to explain the observed movements of radioactivity in the intestine (Fig. 2, B and D and Fig. 3).

Simulated Luminal Distribution of $^{99m}$Tc-DTPA by CAT Model. The CAT model explains movements of intestinal contents with a set of transit times from one compartment to the next. A model with reported transit times (Heikkinen et al., 2012) failed to reproduce the observed movements of $^{99m}$Tc-DTPA (Supplemental Fig. 3). Radioactivity was overestimated in the upper jejunum and underestimated in the lower jejunum in this model. Therefore, appropriate values of transit time were explored through a fitting analysis. Nevertheless, the distribution of $^{99m}$Tc-DTPA was not reproduced in the CAT model, especially for within the lower jejunum and ileum in both fasted and fed states (Fig. 2, E–H). The radioactivity flowed out to the large intestine early, within 2 hours, in all simulations using CAT.

Typical Difference of Estimated Absorption Site in ATOM and CAT Model. Drug distribution in enterocytes is strongly affected by differences in concentrations in the lumen because a drug moves rapidly from the lumen to enterocytes. Therefore, we compared simulated drug concentrations in the enterocytes using ATOM (using variable $D_z$ and fixed $M_t$) and CAT (Fig. 4), in which parameters were optimized to explain the observed intestinal movements of $^{99m}$Tc-DTPA. In this simulation, a drug with a low permeability (approximately 1/50 compared with midazolam) was used to explain a typical difference between the two models because notable differences in drug absorption between the two models are not observed for highly permeable drugs that are absorbed rapidly from the upper jejunum. The CAT model predicted higher drug concentrations in the upper jejunum at 0.5 and 2 hours, but the drug was already considerably transferred from the lower jejunum to the ileum at 6 hours. In contrast,
ATOM predicted that the drug began to move to the lower jejunum even at 0.5 hours and was retained there for 6 hours. The cumulative drug amount reaching the portal vein from each section of the intestine was calculated for these conditions (Fig. 5). These results indicated that most of the drug was absorbed in the upper jejunum in the CAT model and that the absorption occurred mainly in the lower jejunum in ATOM.

Predicting Nonlinear Absorption Ratio of Midazolam and Confirmation of Difference in CYP3A Saturation in Enteroctyes between ATOM and CAT Model. Intestinal metabolizing enzymes and transporters are saturated when drug concentrations are higher than the Michaelis constant in the enterocytes during the absorption process. Therefore, nonlinear dose responses of midazolam F,A,F,G were simulated using the ATOM and CAT model to examine how drug movements in the intestinal lumen may affect oral bioavailability. When simulating results in the fasted state, F,A,F,G values after a midazolam dose of 0.1 mg were estimated to be very similar in both the ATOM and CAT model. The value increased slightly at 1 mg in the CAT model; however, it remained consistent in ATOM (Fig. 6A). At 10 mg, the difference between the F,A,F,G values estimated by the two models was most evident; the observed dose-response of F,A,F,G for midazolam was more similar to the estimation by ATOM than that of the CAT model. Estimated unbound drug concentrations in the enterocytes at 0.16 hours after dosing with 10 mg were compared between the ATOM and CAT model (Fig. 6B). In the CAT model, a higher peak unbound concentration was estimated (14.36 μg/ml) compared with that in ATOM (4.53 μg/ml). Therefore, the absorption window would be narrower in CAT than in ATOM. Since K,m,CYP3A, for midazolam is 1.08 μg/ml (Ando et al., 2015), the degree of saturation would be considerably more extensive in CAT than in ATOM. Additionally, F,A,F,G values of midazolam were estimated to be lower in the fed state than in the fasted state by ATOM (Fig. 6A) because of the lower unbound concentration in the enterocytes in the fed state (Fig. 6B). These results suggest that weaker and more realistic nonlinear pharmacokinetics of drugs may be estimated by ATOM compared with CAT.

Consistency of F,A,F,G Values Predicted by ATOM and TLM. ATOM is an extended model of TLM that maintains various assumptions based on intestinal structures and absorption processes. A previous report by Ando et al. (2015) showed adequate correlation between predicted and calculated F,G values. Therefore, the F,G values predicted using ATOM and TLM were compared to confirm the consistency of the two models. As a result, F,G values predicted using ATOM were within 10% of those predicted using TLM (Fig. 7) for all the drugs examined (Supplemental Table 6). Hence, the results showed the consistency of ATOM and TLM.

Simulation of CYP3A or P-gp–Mediated DIs Using ATOM. ATOM is an expanded model from TLM, but TLM cannot simulate location-dependent DIs because it considers only one absorption site. Clinically, since CYP3A and P-gp are greatly involved in gastrointestinal absorption and many associated clinical DIs have been reported (Kharasch et al., 2004; Galetin et al., 2010; Chu et al., 2018), it is important to be able to accurately predict DIs caused by them. Therefore, we selected midazolam as a typical substrate of CYP3A and digoxin as a typical substrate of P-gp, and we confirmed whether ATOM could explain DIs in combination with the typical perpetrators of CYP3A and P-gp, itraconazole, and clarithromycin. The simulated profiles of the inhibitors were shown in Supplemental Fig. 6. Overall, increases in the plasma concentrations of midazolam and digoxin were simulated using ATOM when perpetrators were administered considering only the intestinal contribution (Fig. 8). However, the increase in midazolam concentrations tended to be overestimated for the 50-mg dose of itraconazole even though the contribution of the liver was not considered. On the other hand, in the higher doses, the elimination phase of midazolam

![Fig. 4. Demonstrative simulation using ATOM and the CAT model of drug concentrations in enterocytes in the small intestine at 0.5 hours (A), 2 hours (B), and 6 hours (C) after oral administration. Solid and broken lines show simulated results by ATOM and CAT model with optimized transit times, respectively. The model compound has the same physiologic and pharmacokinetic parameters (molecular weight, pKₐ values, Kₘ,CYP3A, and unbound fractions in plasma, blood, and enterocytes) as midazolam, except for its apparent permeability in Caco-2 cells and V₅₀,CYP3A, set as 0.02 cm/h (approximately 150 compared with midazolam) and 0 μg/h pmol CYP3A, respectively. The dispersion number and flow rate in the intestine obtained in the analysis shown in Fig. 2A using ⁹⁹mTc-DTPA distribution of subject A in the fasted state as reported by Haruta et al. (2002) were adapted. Dose was set to 1 ng.](https://dmd.aspetjournals.org/cell/atom.png)
was somewhat underestimated, probably due to ignoring the hepatic contribution. Increases in $F_A$ values of digoxin or $F_G$ values of midazolam were estimated as 1.25-fold or 2.15- to 2.58-fold, respectively (Supplemental Table 8).

**Difference of Pharmacokinetics of Midazolam and Digoxin in the Fasted and Fed States.** It was considered that the drug behavior in the small intestine was different between the fasted and fed states from the analysis in Fig. 2. Therefore, using the dispersion number and flow rate obtained in the analysis, the differences in plasma concentrations of midazolam or digoxin were compared between the fasted and fed states, and their significance was evaluated. As a result, both substrates exhibited decreased $C_{\text{max}}$ and delayed time to reach $C_{\text{max}}$ in the fed state (Fig. 9), showing tendencies consistent with the previous reports on the food effects on pharmacokinetics of midazolam and digoxin (Sanchez et al., 1973; Bornemann et al., 1986).

**Discussion**

Precise estimation of drug concentration in the enterocytes is indispensable to consider nonlinear drug absorption and DIs in which intestinal CYP3A and P-gp are involved. Accordingly, precise consideration of drug translocation in the intestinal lumen is necessary because the concentration in the lumen directly affects the concentration in enterocytes. Food intake affects bile secretion, pH, blood flow, and drug translocation in the intestine (Fleisher et al., 1999; Jantratid et al., 2008; Kawai et al., 2011) and often seriously modifies drug absorption with changes in its solubility in the lumen. Haruta et al. (2002) observed changes in drug translocation by monitoring the radioactivity of $^{99m}$Tc-DTPA under the fasted and fed conditions. However, there have been few reports of absorption models to examine the precise drug translocation.

ATOM succeeded in reproducing the distribution of $^{99m}$Tc-DTPA by applying a dispersion model with location-dependent dispersion number and time-dependent intestinal flow (Figs. 2 and 3). The behavior of drugs in the small intestine is quite complex because of its structure and variable motility (Sokolis, 2017). In fact, luminal $^{99m}$Tc-DTPA movement was not simulated in models with fixed dispersion numbers and intestinal flow (Supplemental Fig. 2). In a preliminary analysis, we considered a model with location-dependent dispersion number and flow, but its reproducibility of the drug movement under fed conditions was inferior to that of the adopted model. Therefore, time-dependent intestinal flow was also necessary to explain complex drug translocations in some cases.

Previously, the GITA model explains luminal drug movements precisely using a set of first-order transit rates and lag time (Haruta et al., 2002; Kimura and Higaki, 2002). However, the GITA model lacks the location-dependent physiologic changes in the intestine, whereas CAT and ADAM considers them. In this study, however, the CAT model could not reproduce luminal $^{99m}$Tc-DTPA distribution, regardless of optimizing luminal transit times (Fig. 2, E–H and Supplemental Fig. 2). For this reason, CAT appeared to overestimate $F_A F_G$ of midazolam at lower doses, whereas ATOM predicted the observed values satisfactorily (Fig. 6A). CYP3A expression level is higher in the upper intestine (Paine et al., 1997); thus, the retention of a drug in the upper intestine would be important for intestinal metabolism. CAT estimates a stronger saturation of CYP3A because of its higher concentration in the upper intestine compared with ATOM. The present study implies that...
intestinal absorption models that cannot explain intestinal translocation may lead to a misunderstanding of the nonlinear dynamics of drug absorption.

Intestinal DIs involving CYP3A or P-gp occur because of high drug concentration in the intestine after oral administration (Lilja et al., 2000; Delavenne et al., 2013). This is especially applicable for weak CYP3A inhibitors, since area under the curve increase of them mainly depends on intestinal DIs rather than hepatic ones where drug concentrations may be lower (Yamada et al., 2020). Several CYP3A and P-gp–mediated DIs have been analyzed using PBPK models (Heikkinen et al., 2012; Yamazaki et al., 2019); however, the appropriateness of drug concentrations in the small intestine was not discussed in detail. Currently, DI guidance for the United States, Europe, and Japan only document one formula for estimation of intestinal drug concentration, which divides the dose by the amount of drinking water (250 ml). This is a useful approach for risk management but is far from the concept of PBPK analysis.

In this study, the significances of DIs for midazolam and digoxin cases were explained to a large extent by the intestinal contribution (Fig. 8), suggesting importance of the intestinal DIs. The DI between midazolam and itraconazole was extensively studied, and contributions by the metabolites of itraconazole have been clarified (Isoherranen et al., 2004; Prieto Garcia et al., 2018; Chen et al., 2019), whereas the intestinal contribution was not fully evaluated. In this study, the contribution by the metabolites was not considered because their concentrations in the enterocytes are unknown and would be lower than those in the liver. Nevertheless, in this study, ATOM somewhat overestimates the intestinal interaction by itraconazole. In the future, further detailed studies of DIs are necessary to conclude the precise interactions due to the intestinal contributions.

Regarding pharmacokinetics of digoxin, the contribution of P-gp may not only in the intestine but potentially in the liver and kidney (Liu...
and Sahi, 2016; Yin and Wang, 2016). Therefore, DIs in the liver and kidney should also be considered, which was not achieved in this study. Nevertheless, the analysis of Fig. 8B demonstrated the significance of the intestinal contribution. On the other hand, the predictive performances of P-gp substrates such as cyclosporin and saquinavir were insufficient (Supplemental Table 6). It may be due to relatively high doses of these drugs, thus causing saturation of P-gp. An extensive simulation study of P-gp substrates including verapamil, fexofenadine, talinolol, and digoxin was performed by using TLM, and it was suggested that at higher doses, such as 100 mg, the risk of P-gp-mediated DI would generally be reduced because of saturation of P-gp efflux (Ando et al., 2017).

In addition to the luminal translocation issue, it is necessary to consider the permeability of the basolateral membrane to precisely estimate the drug concentration in enterocytes. In the reported absorption models, ambiguity remains in the descriptions of the basolateral permeability because only one compartment is arranged for the portal blood along with the whole length of the small intestine. If permeation across the basolateral membrane were bidirectional, a drug would be back-secreted from the blood to the lower intestine immediately after absorption begins in the upper intestine. Since no one has reported this phenomenon, it can be assumed that permeation across the basolateral membrane is not truly bidirectional, implying that blood flow–limited absorption cannot be considered by these models, even though the effect of blood flow on drug absorption has been discussed (Winne, 1978; Schulz and Winne, 1987; Chen and Pang, 1997; Pang and Chow, 2012). To solve this problem, the portal blood compartment needs to be separated conceptually by the location of the intestine, as achieved in TLM and ATOM. Additionally, it is necessary to evaluate the permeability of the basolateral membrane separating from that of the apical membrane to incorporate these parameters into the model. This is still a challenging issue, but by using multifunctional cell systems such as induced pluripotent stem cells (Kabeya et al., 2020) and CYP3A4-expressed intestinal cells (Takanaka et al., 2017), it may be possible to discriminate various kinetic intracellular events using selective inhibitors or knockdown techniques.

To precisely reproduce in vivo situations, the PBPK model is composed of complicated structures and parameters, including multiple scaling factors for filling gaps between in vitro and in vivo studies. However, in the case of the intestinal absorption model, if the scaling factor is used to adjust for ambiguity of the absorption site, it should lose its validity for drugs with different absorption sites. In other words, the role of scaling factors is to adjust simply quantitative relationships between in vitro and in vivo. Regarding CYP3A and P-gp substrates, Takano et al. (2016) reported a nonlinear prediction of pharmacokinetics of midazolam successfully with a scaling factor for $V_{\text{max}}$ of CYP3A and explained dose-dependent $F_{\text{a,FG}}$. However, the appropriateness of the scaling factor was not discussed. In this study, the nonlinear absorption of midazolam was predicted using ATOM minimized to only the scaling factors of passive permeability and P-gp expression (Fig. 6A and Supplemental Material). Therefore, we expect that analysis using ATOM would be a useful approach for predicting pharmacokinetics in multiple situations including drug development.

Theoretically, the predicted clearance of an organ pharmacokinetic model is determined by the residence time distribution of the solute and the clearance in the organ (Roberts et al., 1988). ATOM and TLM are designed to be equivalent for these values, so there should be no difference in prediction performance. The performances of ATOM and TLM are comparable to other sophisticated models such as ACAT (Gertz et al., 2010; Ando et al., 2015; Yau et al., 2017). However, their superiority has not been proved yet. Currently, some in vitro parameters for predicting oral availability, including the transport activity by P-gp (Bentz et al., 2013), are variable between experiments, and reliable in vivo $F_{\text{a}}$ and $C_{\text{G}}$ values are insufficient (Hisa-ka et al., 2014). Therefore, further studies are necessary to select the better absorption model.

In the process of drug absorption, dissociation and dissolution are also regulating factors, especially for highly lipophilic and potentially insoluble compounds. These factors are readily influenced by luminal pH, which is lower in the stomach (pH 1.5–5.0) but gradually increases in the intestine (pH 5.0–7.4) (DeSesso and Williams, 2008). At present, ATOM does not include these processes and only considers drug aqueous solutions. Therefore, it is necessary to expand ATOM by incorporating these processes to broaden its applicability.

In conclusion, a newly constructed absorption model, ATOM, could simulate intestinal drug behavior using minimum scaling factors, thereby providing reasonable interpretations of change in drug absorption and of DI mediated by CYP3A and P-gp. In the future, ATOM is expected to be applied to drug development and clinical management.

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Authorship Contributions

Participated in research design: Asano, Sato, Hisaka.
Conducted experiments: Asano, Yoshitomo
Contribute new reagents or analytic tools: Asano, Hozuki, Hisaka
Performed data analysis: Asano, Hisaka.
Wrote or contributed to the writing of the manuscript: Asano, Sato, Kazuki, Hisaka.

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